CLAIMS

1. A method for determining the presence of coliform bacteria in a water sample comprising the steps of:

   a) separating said bacteria from said sample using a first filter means;

   b) culturing said bacteria in a broth comprising nutrients for supporting growth of said bacteria and an inducing agent for inducing enzyme production in said bacteria;

   c) separating said bacteria from said broth using a second filter means;

   d) exposing said bacteria to a lysing agent;

   e) incubating a chemiluminogenic substrate of said enzyme with said enzyme to cause cleavage of said substrate, thereby producing a luminescent product;

   f) initiating light emission by exposing said luminescent product to an enhancing agent; and,

   g) detecting said light emission to thereby determine the presence of said bacteria in said sample.

2. The method of claim 1, wherein said water sample is drinking water.

3. The method of claim 1 or 2, wherein said bacteria are separated from said broth before being exposed to said lysing agent.

4. The method of any one of claims 1 to 3, wherein said bacteria are on said second filter means during exposure to said lysing agent.

5. The method of any one of claims 1 to 4, wherein said light emission is detected by means of a luminometer.

6. The method of claim 5, wherein said luminescent product is on said second filter means during detection of said light emission.

7. The method of claim 6, wherein said second filter means is placed within said luminometer during detection of said light emission.

8. The method of claim 7, wherein said second filter means is flat within said luminometer.

9. The method of any one of claims 1 to 8, wherein said culturing is at a temperature of about 22 to 45 °C for about 2 to 10 hours.

10. The method of any one of claims 1 to 9, wherein said chemiluminogenic substrate comprises 1,2-dioxetane.

11. The method of any one of claims 1 to 10, wherein said enhancing agent comprises quaternary ammonium homopolymer.

12. The method of claim 11, wherein said enhancing agent comprises poly(benzyltributyl)ammonium chloride.

13. The method of any one of claims 1 to 12, wherein said enzyme is ß-D-galactosidase.

14. The method of claim 13, wherein said culturing is at a temperature of about 35 °C for about 5 hours.

15. The method of claim 13 or 14, wherein said inducing agent comprises isopropyl-ß-D-thiogalactopyranoside (IPTG), lactose, or a combination thereof.

16. The method of any one of claims 13 to 15, wherein said substrate comprises 3-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)-tricyclo-[3.3.3.$3^{3,7}$]decan}-4-yl)phenyl β-D-galactopyranoside.

17. The method of claim 16, wherein said substrate is Galacton-Plus™.

18. The method of any one of claims 13 to 17, wherein said enhancing agent is Emerald II™.

19. The method of any one of claims 1 to 12, wherein said enzyme is ß-D-glucuronidase.

20. The method of claim 19, wherein said culturing is at a temperature of about 44.5 °C for about 9 hours.

21. The method of claim 19 or 20, wherein said inducing agent comprises methyl-ß-D-glucuronide (Met-Glu).

22. The method of any one of claims 19 to 21, wherein said substrate comprises sodium 3-(4-methoxyspiro{1,2-dioxetane-3-,2'-(5'-chloro)-tricyclo-[3.3.1.1^{3,7}]decan}-4-yl)phenyl β-D-glucuronate.

23. The method of claim 22, wherein said substrate is Glucuron™.

24. The method of any one of claims 19 to 23, wherein said enhancing agent is Sapphire II™.

25. The method of any one of claims 1 to 24, wherein said lysing agent comprises toluene, successive freeze thaw cycles, a change of pressure, lysozyme, a detergent, octylphenoxypolyethoxyethanol nonionic surfactant, potassium dihydrogen phosphate, polymyxin-B, or a combination thereof.

26. The method of claim 25, wherein said lysing agent comprises octylphenoxypolyethoxyethanol nonionic surfactant, potassium dihydrogen phosphate, and polymyxin-B.

27. The method of any one of claims 1 to 26, wherein said broth further comprises an inhibiting agent for inhibiting the growth of non-target organisms

28. The method of claim 27, wherein said inhibiting agent comprises cefsulodin.

29. A method for determining the quantity of coliform bacteria in a water sample comprising the steps of:

a) separating said bacteria from said sample using a first filter means;

b) culturing said bacteria in a broth comprising nutrients for supporting growth of said bacteria and an inducing agent for inducing enzyme production in said bacteria;

c) separating said bacteria from said broth using a second filter means;

d) exposing said bacteria to a lysing agent;

e) incubating a chemiluminogenic substrate of said enzyme with said enzyme to cause cleavage of said substrate, thereby producing a luminescent product;

f) initiating light emission by exposing said luminescent product to an enhancing agent; and,

g) measuring said light emission to obtain a light measurement corresponding to the quantity of said enzyme to thereby determine the quantity of said bacteria in said sample.

30. The method for determining the quantity of coliform bacteria of claim 29, wherein said water sample is drinking water.

31. The method for determining the presence of coliform bacteria of claim 29 or 30, wherein said bacteria are separated from said broth before being exposed to said lysing agent.

32. The method of any one of claims 29 to 31, wherein said bacteria are on said second filter means during exposure to said lysing agent.

33. The method of any one of claims 29 to 32, wherein said light emission is measured by means of a luminometer.

34. The method of claim 33, wherein said luminescent product is on said second filter means during measurement of said light emission.

35. The method of claim 34, wherein said second filter means is placed within said luminometer during measurement of said light emission.

36. The method of claim 35, wherein said second filter means is flat within said luminometer.

37. The method of any one of claims 29 to 36, wherein said culturing is at a temperature of about 22 to 45 °C for about 2 to 10 hours.

38. The method of any one of claims 29 to 37, wherein said chemiluminogenic substrate comprises 1,2-dioxetane.

39. The method of any one of claims 29 to 38, wherein said enhancing agent comprises quaternary ammonium homopolymer.

40. The method of claim 39, wherein said enhancing agent comprises poly(benzyltributyl)ammonium chloride.

41. The method of any one of claims 29 to 40, wherein said enzyme is ß-D-galactosidase.

42. The method of claim 41, wherein said culturing is at a temperature of about 35 °C for about 5 hours.

43. The method of claim 41 or 42, wherein said inducing agent comprises isopropyl-ß-D-thiogalactopyranoside (IPTG), lactose, or a combination thereof.

44. The method of any one of claims 41 to 43, wherein said substrate comprises 3-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)-tricyclo-[3.3.3.$3^{3,7}$]decan}-4-yl)phenyl β-D-galactopyranoside.

45. The method of claim 44, wherein said substrate is Galacton-Plus™.

46. The method of any one of claims 41 to 45, wherein said enhancing agent is Emerald II™.

47. The method of any one of claims 29 to 40, wherein said enzyme is ß-D-glucuronidase.

48. The method of claim 47, wherein said culturing is at a temperature of about 44.5 °C for about 9 hours.

49. The method of claim 47 or 48, wherein said inducing agent comprises methyl-ß-D-glucuronide (Met-Glu).

50. The method of any one of claims 47 to 49, wherein said substrate comprises sodium 3-(4-methoxyspiro{1,2-dioxetane-3-,2'-(5'-chloro)-tricyclo-[3.3.1.1^{3,7}]decan}-4-yl)phenyl β-D-glucuronate.

51. The method of claim 50, wherein said substrate is Glucuron™.

52. The method of any one of claims 47 to 51, wherein said enhancing agent is Sapphire II™.

53. The method of any one of claims 29 to 52, wherein said lysing agent comprises toluene, successive freeze thaw cycles, a change of pressure, lysozyme, a detergent, octylphenoxypolyethoxyethanol nonionic surfactant, potassium dihydrogen phosphate, polymyxin-B, or a combination thereof.

54. The method of claim 53, wherein said lysing agent comprises octylphenoxypolyethoxyethanol nonionic surfactant, potassium dihydrogen phosphate, and polymyxin-B.

55. The method of any one of claims 29 to 54, wherein said broth further comprises an inhibiting agent for inhibiting the growth of non-target organisms

56. The method of claim 55, wherein said inhibiting agent comprises cefsulodin.

57. A kit for determining the presence or quantity of coliform bacteria in a water sample comprising:

a) a first filter means for separating said bacteria from said sample;

b) a broth for culturing said bacteria comprising nutrients for supporting growth of said bacteria and an inducing agent for inducing enzyme production in said bacteria;

c) a second filter means for separating said bacteria from said broth;

d) a lysing agent for exposure to said bacteria;

e) a chemiluminogenic substrate of said enzyme for incubation with said enzyme to cause cleavage of said substrate, thereby producing a luminescent product;

f) an enhancing agent for initiating light emission upon exposure to said luminescent product; and,

g) wherein said kit is adaptable for use in detecting or measuring said light emission from said luminescent product.

58. The kit of claim 57, wherein said water sample is drinking water.

59. The kit of claim 57 or 58, wherein said bacteria are separated from said broth before being exposed to said lysing agent.

60. The kit of any one of claims 57 to 59, wherein said bacteria are on said second filter means during exposure to said lysing agent.

61. The kit of any one of claims 57 to 60, wherein said light emission is detected or measured by means of a luminometer.

62. The kit of claim 61, wherein said kit includes said luminometer.

63. The kit of claim 61 or 62, wherein said luminescent product is on said second filter means during detection of said light emission.

64. The kit of claim 63, wherein said second filter means is placed within said luminometer during detection or measurement of said light emission.

65. The kit of claim 64, wherein said second filter means is flat within said luminometer.

66. The kit of any one of claims 57 to 65, wherein said culturing is at a temperature of about 22 to 45 °C for about 2 to 10 hours.

67. The kit of any one of claims 57 to 66, wherein said chemiluminogenic substrate comprises 1,2-dioxetane.

68. The kit of any one of claims 57 to 67, wherein said enhancing agent comprises quaternary ammonium homopolymer.

69. The kit of claim 68, wherein said enhancing agent comprises poly(benzyltributyl)ammonium chloride.

70. The kit of any one of claims 57 to 69, wherein said enzyme is ß-D-galactosidase.

71. The kit of claim 70, wherein said culturing is at a temperature of about 35 °C for about 5 hours.

72. The kit of claim 70 or 71, wherein said inducing agent comprises isopropyl-ß-D-thiogalactopyranoside (IPTG), lactose, or a combination thereof.

73. The kit of any one of claims 70 to 72, wherein said substrate comprises 3-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)-tricyclo-[3.3.3.$3^{3,7}$]decan}-4-yl)phenyl β-D-galactopyranoside.

74. The kit of claim 73, wherein said substrate is Galacton-Plus™.

75. The kit of any one of claims 70 to 74, wherein said enhancing agent is Emerald II™.

76. The kit of any one of claims 57 to 69, wherein said enzyme is ß-D-glucuronidase.

77. The kit of claim 76, wherein said culturing is at a temperature of about 44.5 °C for about 9 hours.

78. The kit of claim 76 or 77, wherein said inducing agent comprises methyl-ß-D-glucuronide (Met-Glu).

79. The kit of any one of claims 76 to 78, wherein said substrate comprises sodium 3-(4-methoxyspiro{1,2-dioxetane-3-,2'-(5'-chloro)-tricyclo-[3.3.1.1^{3,7}]decan}-4-yl)phenyl β-D-glucuronate.

80. The kit of claim 79, wherein said substrate is Glucuron™.

81. The kit of any one of claims 76 to 80, wherein said enhancing agent is Sapphire II™.

82. The kit of any one of claims 57 to 81, wherein said lysing agent comprises toluene, successive freeze thaw cycles, a change of pressure, lysozyme, a detergent, octylphenoxypolyethoxyethanol nonionic surfactant, potassium dihydrogen phosphate, polymyxin-B, or a combination thereof.

83. The kit of claim 82, wherein said lysing agent comprises octylphenoxypolyethoxyethanol nonionic surfactant, potassium dihydrogen phosphate, and polymyxin-B.

84. The kit of any one of claims 57 to 83, wherein said broth further comprises an inhibiting agent for inhibiting the growth of non-target organisms

85. The kit of claim 84, wherein said inhibiting agent comprises cefsulodin.

86. A kit for determining the presence of coliform bacteria in a drinking water sample comprising the steps of:

a) separating said bacteria from said sample using a first filter means;

b) culturing said bacteria at a temperature of about 22 to 45 °C for about 2 to 10 hours in a broth comprising nutrients for supporting growth of said bacteria and an inducing agent comprising isopropyl-ß-D-thiogalactopyranoside (IPTG) or methyl-ß-D-glucuronide (Met-Glu) for inducing production of an enzyme in said bacteria;

c) separating said bacteria from said broth using a second filter means; followed by,

d) exposing said bacteria on said second filter means to a lysing agent comprising polymyxin-B;

e) incubating a chemiluminogenic substrate of said enzyme comprising 1,2-dioxetane with said enzyme to cause cleavage of said substrate, thereby producing a luminescent product on said second filter means;

f) initiating light emission by exposing said luminescent product to an enhancing agent comprising quaternary ammonium homopolymer; and,

g) detecting or measuring said light emission using a luminometer by placing said second filter means with said luminescent product within said luminometer to thereby determine the presence or quantity of said bacteria in said sample.